

ESCHERICHIA COLI O157:H7

INACTIVATION OF ZOOBOTIC PATHOGENS DURING STATIC COMPOSTING OF CHICKEN LITTER AND PEANUT HULLS

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During aerobic composting, the primary factor responsible for inactivation of fecal pathogens is heat generated from the metabolic activity of thermophilic microorganisms. Moreover, to ensure inactivation of pathogens at the surface of static compost piles, it is recommended that compost be turned periodically during the first weeks of composting. This safeguard practice, however, is not often implemented in situations where labor and resources are limited. To develop alternative management strategies for these situations, baseline data is needed to determine inactivation profiles of zoonotic pathogens at surface and interior sites of static piles. The fate of zoonotic pathogens [gfp-labeled *Escherichia coli* O157:H7 (Shiga toxin-negative) and *Listeria innocua* and rifampicin-resistant *Salmonella* Typhimurium (vaccine strain)] in the field was monitored at both interior and surface sites of static composting piles composed of chicken litter and peanut hulls. Zoonotic pathogen populations declined by 4-8 log CFU/g within 4 days of composting but were still detectable by enrichment culture. Despite exposures to elevated temperatures, *Salmonella* continued to be detected in interior samples by enrichment for up to 14 days after composting was initiated. In surface samples, the fate of pathogens was dependent on the season and ambient temperature conditions in which composting was conducted. During the summer, *S. Typhimurium*, *E. coli* O157:H7 and *L. innocua* were detected by enrichment only in 3-day, 3-day, and 7-day compost surface samples, respectively. In contrast, 28, 56, and 56 days of composting in the late fall/early winter were required to reduce *S. Typhimurium*, *E. coli* O157:H7, and *L. innocua* populations, respectively, to levels detectable only by enrichment. In conclusion, zoonotic pathogens survived on the surface of unturned static composting piles containing chicken litter for up to 2 months.

INACTIVATION OF *ESCHERICHIA COLI O157:H7* AND *LISTERIA MONOCYTOGENES* IN COW MANURE COMPOSTING SYSTEMS

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Aerobic composting may be applied to manure whereby microbial metabolite degradation of organic matter generates heat for inactivation of pathogens. When equipment and manpower are not available to turn the compost mass and expose all the material to sufficient levels of heat, other management guidelines are needed to assure that pathogen inactivation of surface compost has been achieved. Towards that end, research has been addressing the potential for the initial carbon:nitrogen (C:N) ratio of the compost mixture to affect pathogen inactivation. Using a cow manure, straw, and cottonseed mixture in a laboratory-scale bioreactor, C:N ratio did not significantly affect the time to inactivation of *Listeria monocytogenes*. In contrast, *Escherichia coli* O157:H7 survived for significantly longer periods of time in 40:1 C:N systems than in 30:1 or 20:1 systems despite the fact that the cumulative heat exposure of the former system was much greater than the exposure encountered in the two latter systems. In addition, an escalation in pH to values between 8 and 9 occurred initially for 40:1 C:N systems whereas 20:1 and 30:1 systems experienced an initial decline in pH to values between 5.5 and 6 before climbing to alkaline values (8-9) after 2 days of composting. It is hypothesized that organic acids generated in the acidic stage of 20:1 and 30:1 systems may act in concert with heat to inactivate *E. coli* O157:H7. Such situations may be beneficial to the inactivation of pathogens on the surface of compost piles where temperatures are found to increase only slightly above ambient.

CRYOTOLERANCE, ATTACHMENT, AND RECOVERABILITY OF *ESCHERICHIA COLI O157:H7* AND SELECTED SURROGATES FROM ROMAINE LETTUCE LEAF SURFACES

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A non-pathogenic bacterial species that responds to food processing treatments in a manner equivalent to a foodborne pathogen can potentially be used in actual food processing facilities to evaluate the effectiveness of the process to remove or eliminate the pathogen. Using the surrogate reduces the concerns related to the intentional introduction of pathogens in the food processing environment. It is important that the surrogate behave in a manner similar to that of the pathogenic microorganisms of interest. Surrogates have been evaluated and recommended for

some heat, acidification, and drying procedures used in food processing. There is little work related to having suitable surrogates available to evaluate the fate of pathogens on refrigerated foods. With recent foodborne illness outbreaks related to fresh produce, it is important to have tools which would allow for the evaluation of possible microbial intervention processes on these refrigerated products. The objectives of the study were to determine if non-pathogenic *E. coli* strains could serve as surrogates of *E. coli* O157:H7 for attachment and recoverability studies involving chilled produce and to investigate the effect starvation and cold stress have on the behavior of *E. coli* O157:H7 and selected surrogates.

Five nonpathogenic *E. coli* strains were evaluated for behavior similar to that of *E. coli* O157:H7. The organisms were grown under conditions with minimal nutrients to create starved conditions. To evaluate response to cryotolerance, starved cells were frozen in sterile deionized water at -18°C for 1, 2, 4 and 7 d. After storage at -18°C , control and starved cells were thawed at room temperature and the viable population was determined. To determine whether the possible surrogates attach to lettuce surfaces and can be recovered or removed from the surfaces at a similar rate to *E. coli* O157:H7, romaine lettuce pieces were inoculated with each organism. After 1 hour, pieces were gently rinsed with either sterile deionized water or with chlorinated water.

All *E. coli* strains tested exhibited cryotolerance with less than 1 log CFU/ml decrease over 7 days of storage. In determining the attachment rate to lettuce, it was determined that *E. coli* ATCC 25922 exhibited the greatest attachment rate (79% compared to *E. coli* O157:H7). After chlorine treatment, *E. coli* ATCC 25922 population decreased by a similar rate to that of *E. coli* O157:H7. *E. coli* ATCC 25922 also had similar hydrophobicity compared to *E. coli* O157:H7. Cryotolerance and survival of starved organisms were measured after *E. coli* ATCC 25922 and *E. coli* O157:H7 were held in sterile deionized water for starvation (37°C for 4 hours, 20°C for 24 hours, or 4°C for 7 days). Both stressed *E. coli* O157:H7 and stressed *E. coli* ATCC 25922 exhibited greater cryotolerance than nonstressed control cells. Populations of *E. coli* ATCC 25922 and *E. coli* O157:H7 were reduced by similar amounts (by approx. 99%) after washing with chlorinated water regardless of starvation conditions. *E. coli* ATCC 25922 was found to be a useful surrogate for *E. coli* O157:H7 for studies involving attachment and recoverability of chilled produce.

SILVER NANOROD ARRAY AS A SERS SUBSTRATE FOR *ESCHERICHIA COLI* O157:H7 DETECTION

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The ability to identify pathogens rapidly, nondestructively and distinctively has major benefit to epidemic outbreak and bioterrorism prevention. Surface-enhanced Raman spectroscopy (SERS) has been used as an analytical tool to observe trace amount of chemical and biological molecules due to its capability of giving real-time molecular vibrational information under ambient conditions. As an attempt to meet these needs, we have integrated the silver nanorod arrays substrate fabricated by oblique angle deposition (OAD) technique into a fiber optic surface-enhanced Raman spectroscopy as a portable pathogen sensor for on-site inspection. The substrate consists of a base layer of 500 nm silver film first deposited onto a glass slide and a layer of silver nanorod array with length of $\sim 1\ \mu\text{m}$ deposited by OAD method at a vapor incident angle of 86° . The portable sensor has a sensitivity of 10^{-14} Moles for trans-1,2-bis(4-pyridyl)ethene (BPE). The SERS spectra for *E. coli* O157:H7 and generic *E. coli* were obtained and compared, and distinct spectroscopic fingerprints (Raman peaks around the 735cm^{-1} , 1030cm^{-1} , 1330cm^{-1} , 1450cm^{-1} band) for *E. coli* O157:H7 have been observed. Those SERS spectra and peaks from *E. coli* O157:H7 were reproducible among different batch of substrates and bacteria samples. This study shows that the integrated OAD silver nanorod arrays substrates and fiber Raman system is a potential portable pathogen sensor for on-site food inspection.